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(54) Title: OPTICAL FIBERLESS SENSORS

(57) Abstract

Fiberless optical sensors (plasticized PVC, acrylamide or gold particles) are described having a size ranging from between approximately 1 micrometer and 1 nanometer in diameter. The sensors comprise ionophores useful for the detection of intracellular analytes.

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OPTICAL FIBERLESS SENSORS

FIELD OF THE INVENTION

The invention relates generally to optical fiberless sensors, method of fiberless sensor fabrication and uses of such sensors in cells.

BACKGROUND

The ability of cells, tissues, an organ system and an entire organism to rapidly respond and adapt to exogenous stimuli is a requirement for the maintenance of life. Exposure of a single cell to such stimuli can manifest itself in a variety of ways, including a flux of essential intracellular ions (*i.e.* Na+, K+, Ca++, Cl-, H+), as well as changing oxygen and glucose levels. These changes can trigger additional signaling cascades, ultimately resulting in the recruitment of the appropriate cellular machinery for a response to the stimuli.

Of course, some stimuli are pathogenic to cells. Such stimuli cause a combination of linked and cascading biochemical events leading up to disease and/or cell death. For example, exposure to bacteria, viruses, toxins and toxicants may result in a myriad of intra/extracellular responses, depending on the pathogen or pathogenic agent in question and the route of exposure. The determination and understanding of which of the "downstream" biochemical signals elicited are indicators of physical, chemical or mechanical injury are fundamental to the development of countermeasures and therapy.

Classical biochemical investigations of the toxicologic effects of chemicals on organs and tissues were typically performed on homogenates. This approach reduced complex arrays of cells to a uniform blend. While providing important new information on fundamental mechanisms of toxicology/pharmacology, these studies are limited in their ability to discriminate between cells which are passively or actively involved.

More recent molecular and imaging techniques have improved cellular resolution. However, these newer imaging techniques frequently provide only static

"snapshots" of dynamic cellular processes. Other approaches, while more dynamic, suffer from the fact that the approach alters the cells under study. For example, commercially available fluorescent probes used in the detection of calcium fluxes, chemically bind the moiety in question and potentially alter its homeostasis *in situ*.

Clearly, the most extensive work done intracellularly focused on the direct injection of dyes into the cell. While this method has provided researchers with a simple technique to study cellular processes, it has also proven problematic. For instance, the dye may itself be toxic, or otherwise interfere with the cell chemistry. Another problem is that there is no way to position the dye once it is introduced into the cell. Often, the dye is selectively trapped in some organelles, rather than dispersed evenly throughout the cell.

An additional, critical limitation with the dye injection approach is that the technology is currently limited in selectivity to a small number of analytes. For instance, while there are good dyes for calcium ion detection, there are none for potassium, sodium or chloride.

Fiber optic probes, or optodes, with a polymer sensing element, solve the above problems of dye injection. *See* W. Tan *et al.*, "Submicrometer Intracellular Chemical Optical Fiber Sensors," *Science* 258:778 (1992). These micro-fiberoptic sensors (100-1000 nm) are based on optical grade silica fibers pulled to submicron size. The pulled fiber tips are much less fragile than those of the electrochemical microsensors, which are made from pulled micropipettes. Attached to the tip is a dye-polymer matrix, which is very durable and smooth and runs tightly bound to the tip, even during penetration of biological tissues. The matrix on the end of the fiber often includes several components, such as a chromoionophore, an ionophore, and appropriate ionic additives, all trapped inside a polymer layer, so that no chemicals are free to diffuse throughout the cell. The effects of toxicity of the dyes are thus minimized. Also, the probe can be carefully positioned in the cell, allowing any specific area to be imaged or monitored.

Nonetheless, the fiber optic probes have the significant drawback of being unable to easily monitor more than one location in the cell. For monitoring more than

one location, multiple probes are needed. Due to size constraints, it can prove difficult to position several fibers inside a single cell. Moreover, even the insertion of single fiber sensor can easily damage a cell or short out the cross membrane electrical potential and having several fibers compounds this problem.

Thus, improved methods for studying cells and intracellular analytes are needed. Such improved methods should be amenable to monitoring the cell at more than one location and should have minimal toxicity.

SUMMARY OF THE INVENTION

The invention relates generally to optical fiberless sensors, method of fiberless sensor fabrication and uses of such sensors in cells. The sensors of the present invention are: (1) small enough to enter a single mammalian cell relatively non-invasively, (2) fast and sensitive enough to catch even minor alterations in the movement of essential ions and (3) mechanically stable enough to withstand the manipulation of the sensor to specific locations within the cell.

Importantly, the fiberless sensors of the present invention are non-toxic and permit the simultaneous monitoring of several cellular processes. In one embodiment, the present invention contemplates the use of such fiberless sensors to monitor a single cell exposed to a variety of noxious or trophic stimuli.

The fiberless sensors of the present invention are particularly useful for the direct, real-time, non-invasive, intracellular studies of chemical insults and in elucidation of subcellular mechanisms of action induced by pathogens and related toxins. These sensors are immensely smaller, faster and more sensitive than fiber-optic sensors currently used. The spatially and temporarily highly resolved and highly detailed chemical information gained from using these sensors, greatly speeds up current protocols of research and also leads to new and improved methodologies.

In one embodiment, the present invention contemplates a method comprising: a) providing i) one or more cells, ii) a plurality of fiberless optical sensors, and iii) a means for detecting said sensors; b) introducing said plurality of sensors into said one or more cells; and c) detecting said sensors in said cells with said detecting means.

In another embodiment, the present invention contemplates a method comprising: a) providing i) one or more cells, ii) a plurality of fiberless optical sensors, iii) an exogenous cellular stimulus, and iv) a means for detecting said sensors; b) introducing said plurality of sensors into said one or more cells; c) stimulating said one or more cells with said exogenous cellular stimulus, and d) detecting said sensors in said cells with said detecting means.

In one embodiment, the present invention contemplates a method comprising: a) providing i) first and second preparations of cells, ii) a plurality of fiberless optical sensors, iii) an exogenous cellular stimulus, and iv) a means for detecting said sensors; b) introducing said plurality of sensors into said first and second preparations of cells; c) stimulating said first preparation of cells with said exogenous stimulus, d) detecting said sensors in said cells with said detecting means, and e) comparing the sensors in said first preparation of cells with the sensors in said second preparation of cells.

It is not intended that the present invention be limited by the nature of the cells. Both prokaryotic and eukaryotic cells can be monitored using the sensors of the present invention. Among eukaryotic cells, it is specifically contemplated that the sensors of the present invention are introduced into mammalian cells. All types of mammalian cells are contemplated (e.g. oocytes, epithelial cells, etc.). In some embodiments, cells such as neurons and astrocytes in primary culture are contemplated. Thus, the present invention contemplates generally compositions comprising mammalian cells containing fiberless optical sensors.

In one embodiment, the fiberless sensors are used in the eye. This readily permits monitoring of responses to agents coming in contact with the eye (e.g. gases, aerosols, etc.). In another embodiment, the fiberless sensors are used in the cardiovasculature. This readily permits cardiac monitoring.

It is also not intended that the present invention be limited by the precise composition of the fiberless sensors. The fiberless sensors of the present invention are either solid or semisolid particles ranging in size between approximately 5 micrometer and 1 nanometer in diameter. The ultimate small size is attained by fine grinding and filtering or by micro-emulsion techniques used to form mono-disperse colloidal

particles (rather than nano-fabrication). In one embodiment, the sensor is selected from the group consisting of polymer fiberless sensors, acrylamide fiberless sensors, and metal fiberless sensors.

In one embodiment, the polymer fiberless sensors of the present invention comprise an ionophore, a chromoionophore and a polymer. It is not intended that the present invention be limited to a particular polymer. In one embodiment, the polymer is selected from the group consisting of poly(vinyl chloride), poly(vinyl chloride) carboxylated and poly(vinyl chloride-co-vinyl acetate-co-vinyl alcohol). In a preferred embodiment, the polymer fiberless sensors further comprise an additive and a plasticizer.

In one embodiment, the acrylamide fiberless sensors of the present invention comprise polyacrylamide and a reactive dye. In a preferred embodiment, the acrylamide fiberless sensors further comprise N,N-methylenebi-(acrylamide) and the mixture is polymerized to a gel.

In one embodiment, the metal fiberless sensors of the present invention comprise protein (or peptide) in combination with a metal selected from the group consisting of gold, silver, platinum and alloys thereof. In one embodiment, the protein (or peptide) is dye-labeled (e.g. with FITC).

Regardless of the sensor type (e.g. metal or polymer), the fiberless sensor of the present invention is contemplated to be capable of measuring intracellular analytes, and more particularly, capable of detecting a change in the concentration of intracellular analytes. It is not intended that the present invention be limited to specific analytes. Nonetheless, preferred analytes measured by the sensors of the present invention include, but are not limited to, intracellular ions (*i.e.* Na+, K+, Ca++, Cl-, H+), as well as oxygen and glucose.

It is not intended that the present invention be limited by the manner in which the sensors of the present invention are introduced into cells. In one embodiment, a buffered suspension of fiberless sensors is injected into the sample cell with a commercially-available pico-injector. In another embodiment, the fiberless sensors of the present invention are shot into a cell with a commercially-available particle

delivery system or "gene gun" (such gene guns were developed and are now routinely used for inserting DNA into cells).

In some embodiments, the fiberless sensors of the present invention are positioned with in a cell or remotely steered into a cell, by photon pressure or "laser tweezers". This technique uses an infra-red laser beam which traps the particles. Alternatively, the particles can be moved magnetically, by remotely steering magnetic nanoparticle pebbles (commercially available) into a cell.

It is also not intended that the present invention be limited by the detecting means. In one embodiment, the fiberless sensors of the present invention are addressed by laser beams (rather than fibers), and their fluorescent signals are collected and analyzed by procedures identical to those used for the fiber-tip nanosensors. *See* U.S. Patents Nos. 5,361,314 and 5,627,922 to Kopelman *et al.*, hereby incorporated by reference.

DESCRIPTION OF THE DRAWINGS

Figure 1 schematically shows several embodiments of a detection system for detecting the fiberless optical sensors of the present invention.

Figure 2 schematically shows one given embodiment of a detection system for detecting the fiberless optical sensors of the present invention.

Figure 3 schematically shows the introduction of the optical fiberless sensors of the present invention into cells.

Figure 4 shows the fluorescence spectrum of one embodiment of a fiberless sensor of the present invention when inside and outside a cell.

DEFINITIONS

To facilitate understanding of the invention, a number of terms are defined below.

An "allergic reaction" is any abnormal or altered reaction to an antigen (or "allergen"). Typically this reaction is characterized by hypersensitivity of the body to specific substances, whether protein, lipid or carbohydrate in nature. Allergic reactions

may be local, such as contact dermatitis, or may be systemic, such as anaphylaxis. Among allergic diseases, bronchial asthma is one of the most significant. In most urban hospitals, it is the leading cause of admission of children. Current medical practice accepts asthma in afflicted individual to be an unavoidable, incurable illness.

The term "analyte" is intended to comprise any substance within a cell. Analytes of particular interest include (but are not limited to) intracellular ions (*i.e.* Na+, K+, Ca++, Cl-, H+), as well as oxygen and glucose.

The term "chemical reaction" means reactions involving chemical reactants, such as inorganic compounds.

The phrase "exogenous cellular stimulus" means a stimulus exogenous to a cell that is capable of stimulating the cell. By "stimulating the cell" is meant that the status of the intracellular analytes of the cell is changed (e.g. the concentration is changed).

Such stimuli include, but are not limited to a variety of noxious, pathogenic and trophic stimuli. In one embodiment, the stimulus is a toxic agent (or "toxicant"). In another embodiment, the toxic agent is a biological toxin.

It is not intended that the present invention be limited to particular toxins. For example, prokaryotes are a known source of a variety of toxins. Among species of bacteria, the most notorious toxin sources are certainly *Clostridum botulinum* and *Clostridium parabotulinum*. The species produce the neurogenic toxin known as botulinus toxin. While a relatively rare occurrence in the United States, involving only 355 cases between 1976 and 1984 (K.L. MacDonald *et al.*, *Am J. Epidemiology* 124, 794 (1986)), the death rate due to the botulism toxin is 12% and can be higher in particular risk groups. C.O. Tacket *et al.*, *Am. J. Med.* 76, 794 (1984).

Many other bacteria produce protein toxins of significance to humans, including *Bacillus anthracis, Bordetella pertussis (diptheria), Pasteurella pestis, Pseudomonas aeruginosa, Streptococcos pyrogenes, Bacillus cereus, E. coli, Shigella, Staphylococcus aureus, Vibrio cholerae,* and *Clostridium tetani.* Thorne and Gorbach, Pharmacology

of Bacterial Toxins, In: International Encyclopedia of Pharmacology and Therapeutics, F. Dorner and J. Drews (eds.), Pergamon Press, Oxford (1986), pp. 5-16.

"Initiating a reaction" means causing a reaction to take place. Reactions can be initiated by any means (e.g. heat, wavelengths of light, addition of a catalyst, etc.)

The term "microorganism" as used herein means an organism too small to be observed with the unaided eye and includes, but is not limited to bacteria, viruses, protozoans, fungi, and ciliates.

The term "bacteria" refers to any bacterial species including eubacterial and archaebacterial species.

The term "virus" refers to obligate, ultramicroscopic, intracellular parasites incapable of autonomous replication (*i.e.*, replication requires the use of the host cell's machinery).

A "solvent" is a liquid substance capable of dissolving or dispersing one or more other substances. It is not intended that the present invention be limited by the nature of the solvent used.

DESCRIPTION OF THE INVENTION

The invention relates generally to optical fiberless sensors, method of fiberless sensor fabrication and uses of such sensors in cells. The fiberless sensors allows for direct insertion, without the need for buffering solutions, which could change the analyte contents inside a cell. The fiberless sensors have the advantages of fiber micro-optodes, but are much smaller, less invasive and totally encapsulated by the cell or even by one of its organelles. They also have the potential for multiple analyte measurements and multiple positioning inside a single cell.

The fiberless sensors of the present invention comprise one ore more active sensor molecule (e.g. calcium green) that is embedded in an inert host (e.g., acrylic polymer). These sensors have numerous advantages compared with individual molecular tags (e.g., calcium green), including but not limited to: (1) No toxicity or interference with cell chemistry; (2) No selective sequestration in subcellular organelles; (3) No lipophilicity requirement for "smuggling" the molecule across the

membrane; (4) No ion buffering; (5) No quenching of fluorescence by heavy metals; (6) No dye loss through leakage; (7) No alteration of fluorescence by sample viscosity; (8) No poor resolution at cell edges. Most importantly, (9) the fiberless sensors allow parallel processing of multiple chemical analytes (most fluorescent tags do not allow this due to mutual interference by spectral overlap, fluorescence quenching and chemical interactions).

A. Optical Fiberless Sensors

The present invention contemplates fiberless sensors or Probes Encapsulated By BioListic Embedding (PEBBLEs). While a variety of such fiberless sensors are contemplated (including but not limited to metal particles), the preferred fiberless sensors of the present invention are finely ground or formed particles comprising polymer matrices that are incorporated with fluorescent dyes, ionophores, and/or other components. These sensors, with sizes ranging from the submicrometer to micrometer, can be made from any polymer matrix. A preferred polymer matrix comprises plasticized poly(vinyl chloride).

The fiberless sensors are particularly suitable for chemical analysis in mammalian cells, by inserting the sensors into the cell, and monitoring remotely. The sensor particles or beads can be dispersed in buffer solution and pico-injected into a cell. The particles can be monitored singly, in groups located at different positions, or several different kinds can be injected for simultaneous measurements of several distinct intracellular ion or small molecule concentrations.

The fiberless sensors circumvent many of the problems associated with optical fiber sensors. While the fiberless sensors of the present invention can be made of the same polymer matrix as that used on the end of an optode, they do not have the fiber size constraints and the associated consequences for the cell. A plurality of fiberless sensors of the present invention can be injected at one time, giving a means for simultaneous measurements. This can even be done with a single penetration, and the cell wall can be allowed to recover. The polymer matrices have been shown to have

very little effect on the cell itself (such as toxicity), and can be left inside for the lifetime of the cell.

B. Sensor Fabrication

In one embodiment, the fiberless sensor of the present invention comprises several components, such as a chromoionophore, an ionophore, and appropriate ionic additives, all trapped inside a polymer layer, so that no chemicals are totally free to diffuse throughout the cell. The precise composition depends on which analyte (or analyte ion) is sought to be measured.

In one embodiment, the particle is prepared for each analyte by selecting one ionophore (30 mmol/kg), one chromoionophore (15 mmol/kg), one additive (15 mmol/kg), one polymer (33 wt %) and one plasticizer (66 wt %). This mixture should then be dissolved in solvent. The preferred solvent is freshly distilled THF (200 mg mixture in 5 mL THF). The solution can then be coated onto polystyrene spheres, and ground in liquid nitrogen.

It is not intended that the present invention be limited by the nature of the ionophore and/or chromoionophore. For *e.g.*, Porphyrins (from Aldrich chemical), are dyes that can be used for oxygen sensors; Calixarenes and Cobyrinates are examples of ionophores that can be used for sodium and nitrite ions respectively. Other illustrative examples (allowing for mixing and matching) are provided in the tables below (all of the chemicals are commercially available; most are available from Fluka Chemical Corp, Ronkonkoma, NY).

C. Uses Of The Present Invention

The invention will be useful for, among other things, in the identification of cellular and subcellular responses which lead to disease. The fields in which the fiberless optical sensors of the present invention will find application are vast, and include basic research, diagnosis, and treatment of disease. Direct benefits to humans and the environment include the development of new drugs, understanding the synergistic response to complex mixtures of pollutants, and prevention of

TABLE 1 - ILLUSTRATIVE IONOPHORES

Analyte Ion	Ionophore	Alternate Name
Primary amines	Amine Ionophore I	
Ammonium	Ammonium Ionophore I	
Barium	Barium Ionophore I	
Cadmium	Cadmium Ionophore I	ETH 1062
Calcium	Calcium Ionophore I	ETH 1001
	Calcium Ionophore II	ETH 129
	Calcium Ionophore III	Calcimycin
	Calcium Ionophore IV	ETH 5234
Carbonate	Carbonate Ionophore I	ETH 6010
	Carbonate Ionophore II	ETH 6019
	Carbonate Ionophore III	ETH 6022
	Carbonate Ionophore IV	
Cesium	Cesium Ionophore I	
Chloride	Chloride Ionophore I	
	Chloride Ionophore II	ETH 9009
Copper (II)	Copper (II) Ionophore I	o-XBDiBDTC
Hydrogen	Hydrogen Ionophore I	
	Hydrogen Ionophore II	ETH 1907
	Hydrogen Ionophore III	
	Hydrogen Ionophore IV	ETH 1778
Hydrogen Sulfite	Hydrogen sulfite Ionophore I	ETH 5444
Lead	Lead Ionophore I	ETH 322
	Lead Ionophore II	MBDiBDTC
	Lead Ionophore III	ETH 5435
	Lead Ionophore IV	
	Lead Ionophore V	15-Crown-5
Lithium	Lithium Ionophore I	ETH 149
	Lithium Ionophore II	ETH 1644
	Lithium Ionophore III	ETH 1810
	Lithium Ionophore IV	ETH 2137
	Lithium Ionophore V	12-Crown-4
	Lithium Ionophore VI	6,6-Dibenzyl-14-crown-4
	Lithium Ionophore VII	
	Lithium Ionophore VIII	
Magnesium	Magnesium Ionophore I	ETH 1117
	Magnesium Ionophore II	ETH 5214
	Magnesium Ionophore III	ETH 4030
	Magnesium Ionophore IV	ETH 7025

TABLE 1 (CONTINUED)

Analyte Ion	Ionophore	Alternate Name
Nitrite	Nitrite Ionophore I	*
	Nitrite Ionophore II	
	Nitrite Ionophore III	
Potassium	Potassium Ionophore I	Valinomycin
	Potassium Ionophore II	
	Potassium Ionophore III	BME-44
Silver	Silver Ionophore I	
	Silver Ionophore II	MAO
	Silver Ionophore III	
	Silver Ionophore IV	
Sodium	Sodium Ionophore I	ETH 227
	Sodium Ionophore II	ETH 157
	Sodium Ionophore III	ETH 2120
	Sodium Ionophore V	ETH 4120
	Sodium Ionophore VI	
	Sodium Ionophore X	
Uranyl	Uranyl Ionophore I	ETH 295
Zinc	Zinc Ionophore I	

* (Cyanouqua-cobyrinic acid hepatokis 2 - phenylethyl ester)

TABLE 2 - ILLUSTRATIVE CHROMOIONOPHORES

Chromoionophores	Alternate Name
Chromoionophore I	ETH 5294
Chromoionophore II	ETH 2439
Chromoionophore III	ETH 5350
Chromoionophore IV	ETH 2412
Chromoionophore V	
chromoionophore VI	ETH 7075
Chromoionophore XI	ETH 7061

TABLE 3 - ILLUSTRATIVE ADDITIVES

Cesium tetrakis(3-methylphenyl)borate
Potassium tetrakis[3,5-bis(trfluoromethyl)phenyl]borate
Potassium tetrakis(4-chlorophenyl)borate
Sodium tetrakis[3,5-bis(trfluoromethyl)phenyl]borate
Sodium tetrakis(4-fluorophenyl)borate Dihydrate
Sodium tetraphenylborate
Tetrabutylammonium tetraphenylborate
Tetradodecylammonium tetrakis(4-chlorophenyl)borate
Tetraheptylammonium tetraphenylborate
Tetraphenylphosphonium tetraphenylborate

TABLE 4 - ILLUSTRATIVE PLASTICIZERS

Plasticizors	Alternate Name
Benzyl ether	
Benzyl 2-nitrophenyl ether	
Bis(1-butylpentyl) adipate	
Bis(1-butylpentyl) decane-1, 10-diyl diglutarate	
Bis(2-ethylhexyl) adipate	
Bis(2-ethylhexyl) sebacate	DOS
1-Chloronaphthalene	
Chloroparaffin	
1-Decanol	
Dibutyl phthlate	
Dibutyl sebacate	
Dibutyltin dilaurate	
1,2-Dimethyl-3-nitrobenzene	
Dioctyl phenylphosphate	
Dipentyl phthalate	
1-Dodecanol	
Dodecyl 2-nitrophenyl ether	ETH 217
[12(4-Ethylphenyl)dodecyl] 2-nitrophenyl ether	ETH 8045
2-Fluorophenyl 2-nitrophenyl ether	
1-Hexadecanol	
10-Hydroxydecyl butyrate	ETH 264
2-Nitrodiphenyl ether	
2-Nitrophenyl octyl ether	o-NPOE
2-Nitrophenyl pentyl ether	
1-Octadecanol	
Octyl [2-(trifluoromethyl)phenyl] ether	ETH 5406
5-Phenyl-1-pentanol	
1-Tetradecanol	
Tetraundecyl benzhydrol-3,3',4,4'-tetracarboxylate	ETH 2112
Tetraundecyl benzophenone-3,3',4,4'-tetracarboxylate	ETH 2041
Tributyl phophate	
Trioctylphosphine oxide	
Tris(2-ethylhexyl) phosphate	
Tris(2-ethylhexyl) trimellitate	

developmental and degenerative disorders. Fiberless optical sensors will find application in any setting where current techniques assess whole organism (including but not limited to intraembryonic applications) or whole cell chemistry in an attempt to elucidate specific mechanisms of toxicity. The fiberless optical sensors will bring to light an entirely new level of detail not previously available.

1. **Elucidate Responses to Toxicants**

The responses to toxicants can be elucidated in developmental and adult models of toxicity *in vitro, in utero, in vivo*, or in slice single cell suspensions, mono or bi-layers, colonies. The types of toxicants that can be evaluated include bacterial, viral, prion, fungal, protozoan, plant, anthropogenic (pesticides, complex organic compounds used in manufacturing and their emissions and discharges, oxidants, environmental degradation products of natural and anthropogenic chemicals), synthetic and natural nutritional supplements, electromagnetic radiation, contact media (ingestion, inhalation, dermal, mucosal).

The fiberless sensors of the present invention can be used with success to assist in the identification of structure-activity relationships and chemical nature of toxicants inside and outside the cell. This information will provide predictive abilities for a myriad of applications including pesticide design, understanding the biochemical role of CFC substitutes on plants and animals prior to their mass production and release, and ultimately even setting regulations for toxicologically-significant levels of pollutants in air, water, and food.

In one embodiment, the present invention contemplates utilizing the intracellular analyte response pattern to identify identify the pathogenic/toxic agent. That is to say, the response to one type of toxic agent (or even a particular agent within a class of toxic agents) can be monitored for the pattern of intracellular analyte changes. Such a pattern becomes a "fingerprint" for exposure to that particular agent, allowing for more rapid treatment of the individual and/or prompt removal of the individual from the source or "zone" of exposure.

2. **Development of Diagnostic Tools and Treatment of Disease**

As noted above, the fiberless sensors of the present invention can be used to measure any alteration in endogenous analytes of any cell. The present invention specifically contemplates transcutaneous monitoring (*e.g.* ear, skin) as well as continuous flow monitoring of cells in culture, organotypic culture, organ slices, isolated perfused organs, organs *in situ*, and whole animal monitoring.

The present invention contemplates that the fiberless sensors of the present invention can be used as diagnostic tools for earlier intervention (*i.e.* earlier than currently available) and treatment of disease. Examples of where early intervention is important include, but are not limited to, allergic responses and septic shock.

Allergic Reponses

In one embodiment, the fiberless sensors are used to detect allergic responses. In this regard, inhalation of allergens by sensitized subjects typically results in an early phase response characterized by bronchoconstriction within 10 minutes of inhalation, reaching a maximum within 1 to 2 hours. In some subjects, the airway narrowing recurs after 3 to 4 hours (i.e., late phase), reaching a maximum during the next few hours. P.M. O'Byrne *et al.*, *Am. Rev. Respir. Dis.* 136:740 (1987). This late phase is thought to be due to the cellular phase of inflammation. F.E. Hargreave *et al.*, *Eur. J. Respir. Dis.* 69 (Suppl 147): 16 (1986). P.M. O'Byrne, *Chest* 90:575 (1986). J. Dolovich *et al.*, *J. Allergy Clin. Immunol.* 83 (Suppl):521 (1987).

The present invention contemplates the use of fiberless optical sensors in the relevant cells of the allergic individual to allow for detection of an allergic response within seconds or (at most) minutes after exposure to an allergen. This allows for earlier intervention and treatment.

Sepsis and Septic Shock

Sepsis is a major cause of morbidity and mortality in humans and other animals. It is estimated that 400,000-500,000 episodes of sepsis resulted in 100,000-

175,000 human deaths in the U.S. alone in 1991. Sepsis has become the leading cause of death in intensive care units among patients with non-traumatic illnesses. [G.W. Machiedo *et al.*, *Surg. Gyn. & Obstet.* 152:757-759 (1981).] It is also the leading cause of death in young livestock, affecting 7.5-29% of neonatal calves [D.D. Morris *et al.*, *Am. J. Vet. Res.* 47:2554-2565 (1986)], and is a common medical problem in neonatal foals. [A.M. Hoffman *et al.*, *J. Vet. Int. Med.* 6:89-95 (1992).] Despite the major advances of the past several decades in the treatment of serious infections, the incidence and mortality due to sepsis continues to rise. [S.M. Wolff, *New Eng. J. Med.* 324:486-488 (1991).]

Sepsis is a systemic reaction characterized by arterial hypotension, metabolic acidosis, decreased systemic vascular resistance, tachypnea and organ dysfunction. Sepsis can result from septicemia (*i.e.*, organisms, their metabolic end-products or toxins in the blood stream), including bacteremia (*i.e.*, bacteria in the blood), as well as toxemia (*i.e.*, toxins in the blood), including endotoxemia (*i.e.*, endotoxin in the blood). Septicemia and septic shock (acute circulatory failure resulting from septicemia often associated with multiple organ failure and a high mortality rate) may be caused by a number of organisms.

The systemic invasion of microorganisms presents two distinct problems. First, the growth of the microorganisms can directly damage tissues, organs, and vascular function. Second, toxic components of the microorganisms can lead to rapid systemic inflammatory responses that can quickly damage vital organs and lead to circulatory collapse (*i.e.*, septic shock) and oftentimes, death.

The present invention contemplates the use of fiberless sensors in patients at risk for septic shock. Introduction of the sensors in the patient's cells (*e.g.*, lymphocytes) allows for earlier detection of toxin exposure and thus, prompt intervention.

3. Imaging

The fiberless sensors of the present invention are ideal for simultaneous real-time monitoring of many (e.g. six) chemical analytes as well as acting as parallel processors. They may also be used for spatially resolved information. The spatial distribution of the fiberless sensors in a cell can be observed with Laser Scanning Confocal Microscopy(LSCM). Of interest is whether any segregation occurs in one location of the cell preferentially over others. Cellular component labeling, 3-D imaging and visualization, can be done at an Imaging laboratory. Rearrangement of fiberless sensors following injection can be monitored by periodically acquiring a series of 3-D LSCM images. High resolution (100 x NA 1.4) images can be obtained to determine analyte concentration and changes can be determined with sub-section imaging of sensor locations. Several (2,3, or more) individual sensor locations can be identified within the small localized area of the cell where the pebbles are injected. Only those locations are then imaged confocally in order to follow in time (scale of hours) analyte changes after a biochemical perturbation. Significant photobleaching from such occasional scans, are not anticipated [i.e. the extremely small (micron or submicron) photoexcited region limits photo-damage to that region and enables fast diffusive replenishment from the dark environment]. Also, images can be obtained simultaneously at two or more wavelengths using an electronically tunable filter on a time scale of hours.

Utilizing the fiberless optical nanosensors of the present invention, one can get a chemical video for a single analyte (e.g. calcium), analogous to a black and white movie, or a chemical video of a list of chemical analyte (e.g., calcium, sodium, potassium, chloride, oxygen and pH), analogous to a color video or, alternatively, to six single color videos, each taken with a different narrow-band optical filter.

For such monitoring, the cell is placed under a microscope objective lens (see Figure 1), and may be immobilized by a micropipette (standard technique). One or several fiberless sensors are introduced into the cell(s) and are addressed optically by laser beams.